

IDENTIFICATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS FROM RED GRAPE POMACE

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Abstract: The wine industry generates a great amount of waste every year, thus its valorization is of most importance. This study uses red, fermented pomace from Cabernet Sauvignon and Feteasca Neagra cultivars. The phenolic compounds were extracted using four different extractions and the content of total polyphenols was determined using a spectrophotometrical method. Several phenolic compounds were analyzed using an HPLC method. The Cabernet Sauvignon pomace had the highest content of total polyphenols and total phenolic compounds analyzed and of quercetin, rutin, ferulic acid and resveratrol, while the Feteasca Neagra pomace had the highest content of gallic acid, syringic acid, cinamic acid and (+) - catechin. The caffeic acid and the chlorogenic acid were not found in any of the analyzed pomaces. These pomaces could be used in the food industry as functional ingredients.

Keywords: by-products, grapes, HPLC, total polyphenols, winemaking waste

INTRODUCTION

Vitis vinifera L. is an important crop in Romania and worldwide. About 80 % of the annual production is used in the winemaking process [1]. The winemaking industries produce millions of tons of residues after fermentation. These are represented by grape pomace that is comprised of skins, seeds and stems of grapes [1].

The grape pomace has been studied in the last few years because it is a by-product that still contains significant amounts of several polyphenolic compounds [2 – 4]. A great interest was enounced for the identification and quantification of phenolic compounds because they can be a cheap source of compounds with antioxidant potential and health benefits [5 – 7]. Polyphenols can provide preventive actions against cardiovascular and neurodegenerative diseases, diabetes and cancer [8 – 11]. Therefore, it is recommendable that the by-products from the winemaking industry to be used in the food industry as functional ingredient.

MATERIAL AND METHODS

Sample preparation

The samples used were red, fermented pomaces from two different cultivars from Transylvania region wineries. They consisted of seeds, stems and skins of grapes from Cabernet Sauvignon and Feteasca Neagra cultivars.

The pomaces were dried in air stream, ground on a domestic mill and stored at -20 °C until analysis. At the time of the analysis the samples of pomaces were defrosted and dried in air stream until they reached constant weight.

Extractions

Extraction 1: The extraction was performed by adding 10 mL of methanol to 500 mg of dried pomace powder and then the mixture was brought to boiling point. After cooling and filtering, 10 mL 2 N hydrochloric acid solution was added, and the mixture was heated on a water bath for 30 minutes using a refluxing system. After cooling and filtering, a funnel extraction was performed, using ethyl acetate as solvent. The resulted extract was dried using a rotary evaporator system and the residue was dissolved into 10 mL methanol before analysis.

Extraction 2: The extraction was performed by adding 10 mL of methanol to 500 mg dried pomace powder. It was covered and put in an ultrasound bath at 40 °C for 30 minutes. The mixture was centrifuged at 5000 rpm for 10 minutes, and the resulting supernatant layer was filtered and brought to dryness using a rotary evaporator system. The residue was dissolved into 10 mL methanol before analysis.

Extraction 3: This extraction was performed like extraction 2. The solvent used for the extraction was methanol and purified water 70:30 (V/V) [12].

Extraction 4: This extraction was performed like extraction 2. The solvent used for the extraction was methanol, purified water and 0.12 M hydrochloric acid solution 70:29:1 (V/V/V) [13].

Analysis

Total polyphenols: The content in total polyphenols was determined using the Folin-Ciocalteu method. The results were expressed as mg gallic acid equivalents (GAE)/mL using a calibration curve. The determination was made spectrophotometrically, by using a Thermo Scientific Evolution 300 spectrophotometer, at 760 nm.

Phenolic profile: The quantitative and qualitative analysis of phenolic compounds was carried out on a Agilent Technologies 1200 series HPLC system, equipped with degasser, quaternary pump, diode array detector, thermostated autosampler and thermostated column compartment. The column used was Zorbax Eclipse Plus C18 (250 mm x 4.6 mm i.d. x 5 μ m) provided from Agilent Technologies (United States of America), at controlled temperature of 25 °C. The elution was performed using purified water (mobile phase A), methanol (mobile phase B) and purified water and glacial acetic acid 96:4 (V/V) (mobile phase C), provided from Merck Millipore (Germany). The gradient program was used as follows: 0 min: 15 % B and 85 % C, 15 min: 75 % A and 25 % B, 20 min: 15 % A and 85 % B, 40 min: 40 % A and 60 % B, 45 min: 5 % A and 95 % B, 55 min: 5 % A and 95 % B, 60 min: 85 % A and 15 % B and 70 min: 85 % A and 15 % B. The flow rate program was used as follows: 0 min: 0.5 mL·min⁻¹ and 15 to 70 min: 0.8 mL·min⁻¹. The injection volume was 5 μ L and the detection was performed at 280, 303, 330 and 360 nm [12, 13]. The standards of gallic acid, ferulic acid, syringic acid, cinnamic acid, chlorogenic acid, caffeic acid, (+)-catechin, resveratrol, quercetin, rutin were purchased from Sigma Aldrich, at HPLC purity.

RESULTS AND DISCUSSION

The determination of total polyphenols reveals the highest quantity of these compounds being extracted using extraction 4 (E4), followed by extraction 3 (E3), extraction 2 (E2), and the smallest by using extraction 1 (E1) (Figure 1).

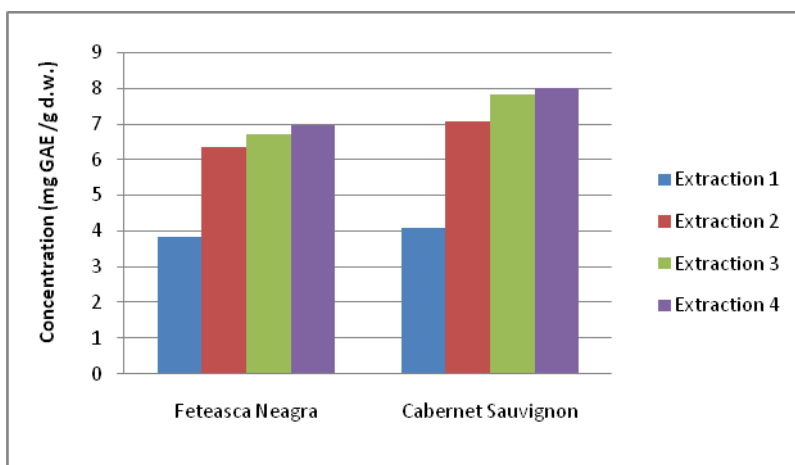
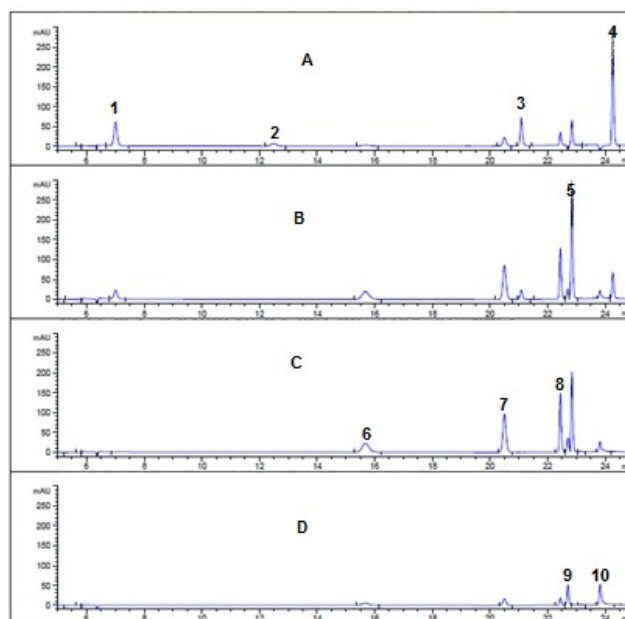


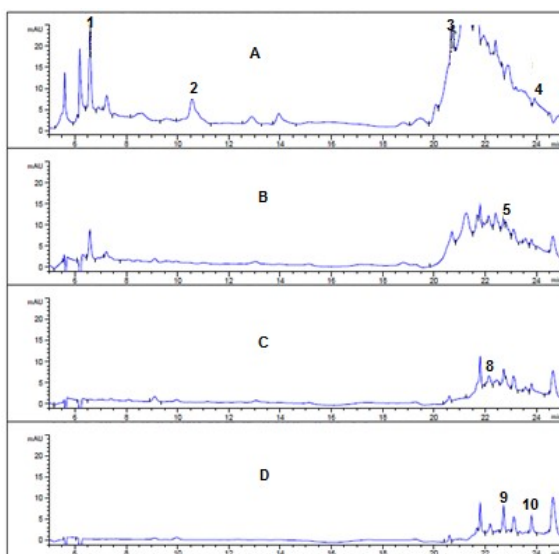
Figure 1. Determination of total polyphenols

The identification of several phenolic compounds is presented in Table 1, thus it is shown that all the phenolic compounds that were found by analyzing the pomaces

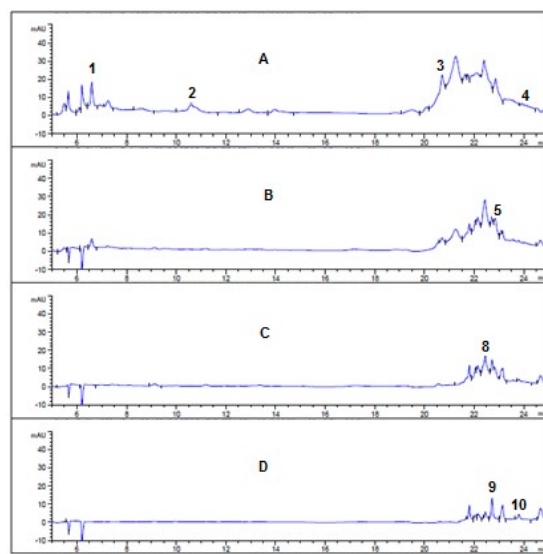
samples were identified regardless of the extraction used. The caffeic acid and the chlorogenic acid couldn't be identified by any performed extraction (Figure 2; Table 1).



Chromatogram of the mixture of standards



Chromatogram of the phenolic compounds analyzed from the Feteasca Neagra pomace, E4



Chromatogram of the phenolic compounds analyzed from the Cabernet Sauvignon pomace, E4

Figure 2. Chromatograms of the analysis of the phenolic compounds

A- 280 nm, B-303 nm, C-330 nm, D-360 nm

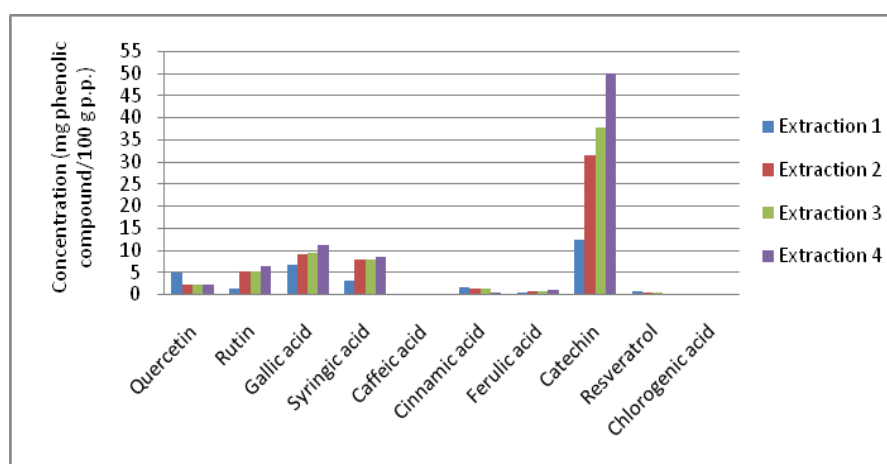
1- Gallic acid, 2- (+)-Catechin, 3- Syringic acid, 4- Cinnamic acid, 5- Resveratrol, 6- Chlorogenic acid, 7- Caffeic acid, 8- Ferulic acid, 9- Rutin, 10- Quercetin

Table 1. Identification of the phenolic compounds analyzed

Compound	Cabernet Sauvignon				Feteasca Neagra			
	E1	E2	E3	E4	E1	E2	E3	E4
Gallic acid	+	+	+	+	+	+	+	+
(+)-Catechin	+	+	+	+	+	+	+	+
Syringic acid	+	+	+	+	+	+	+	+
Cinnamic acid	+	+	+	+	+	+	+	+
Resveratrol	+	+	+	+	+	+	+	+
Caffeic acid	-	-	-	-	-	-	-	-
Chlorogenic acid	-	-	-	-	-	-	-	-
Ferulic acid	+	+	+	+	+	+	+	+
Rutin	+	+	+	+	+	+	+	+
Quercetin	+	+	+	+	+	+	+	+

To extract the phenolic compounds with the best yield possible, the comparison between the retrieved quantities using the four extractions was made for both pomace cultivars. Thus, for quercetin, resveratrol and cinnamic acid the best extraction yield was determined for E1, followed by E2, E3 and E4 and for rutin, gallic acid, syringic acid, ferulic acid and (+)- catechin was for E4, followed by E3, E2 and E1 (Figures 3 and 4).

The quantification of the phenolic compounds of the pomace from the Feteasca Neagra cultivar shows the quantity of (+)- catechin greater than 45 mg / 100 g pomace powder (p.p.). The quantities of rutin, gallic acid and syringic acid greater than 5 mg / 100 g p.p. and the quantities of quercetin, cinnamic acid, ferulic acid and resveratrol lower than 5 mg /100 g p.p. For this assessment the largest quantity of each phenolic compound was enounced using the best yield extraction for each compound (Figure 3).

**Figure 3.** The quantitative determination of phenolic compounds from pomace from the Feteasca Neagra cultivar

Regarding the Cabernet Sauvignon cultivar, the highest quantity of all the phenolic compounds analyzed was of (+) - catechin, of more than 40 mg / 100 g p.p. The quantity of rutin was determined to be greater than 10 mg / g p.p. and of quercetin,

gallic acid and syringic acid, greater than 5 mg / 100 g p.p. The quantities of cinnamic acid, ferulic acid and resveratrol were determined to be less than 5 mg / 10 g p.p. (Figure 4).

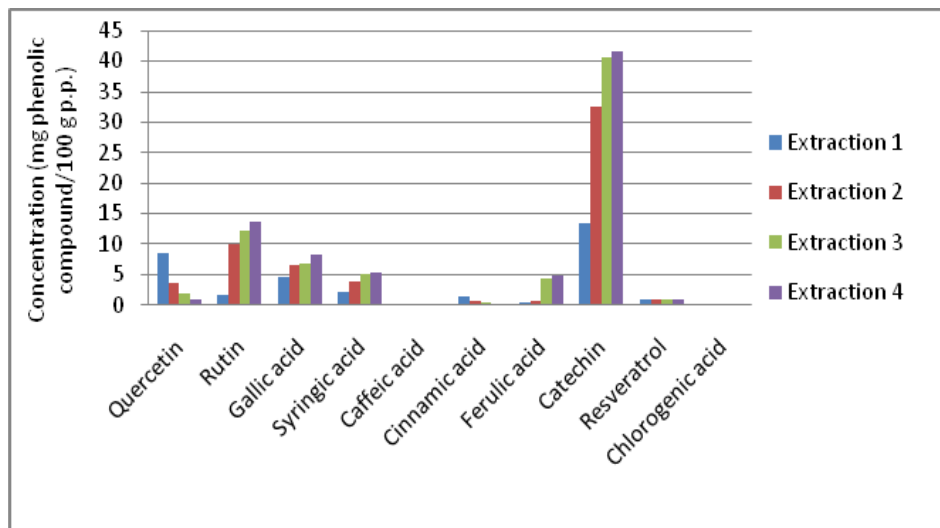


Figure 4. The quantitative determination of phenolic compounds from pomace from the Cabernet Sauvignon cultivar

Both red pomaces had the same content in phenolic compounds regarding the identification of them, but the quantities of these compounds have varied depending on the pomace cultivar (Figure 5).

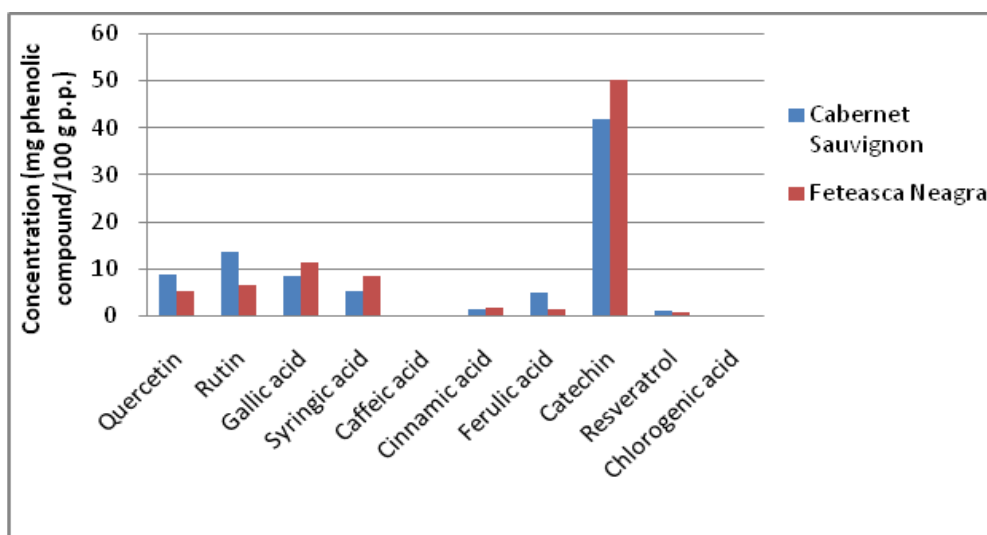


Figure 5. Comparison of phenolic compounds quantities from red pomaces

Regarding the phenolic compounds analyzed, the highest amounts of gallic acid, syringic acid, cinnamic acid and (+) - catechin were determined for the pomace from the Cabernet Sauvignon cultivar and quercetin, rutin, ferulic acid and resveratrol for Feteasca Neagra cultivar pomace (Figure 5).

CONCLUSIONS

This study was based on the identification and quantification of several phenolic compounds extracted from by-products from the winemaking industry. Among the four extraction methods used, only two have proven their efficiency.

For the extraction of the total polyphenols, the yield was determined by using extraction method number 4. This extraction has proven to be the best for rutin, gallic acid, syringic acid, ferulic acid and (+) - catechin. Quercetin, cinnamic acid and resveratrol were best extracted by using extraction method number 1.

During these analyses, chlorogenic acid and caffeic acid couldn't be identified in none of the red pomaces samples.

The highest quantity of total polyphenols as well as quercetin, rutin, ferulic acid and resveratrol were determined in the Cabernet Sauvignon pomace. The Feteasca Neagra pomace had the highest content of gallic acid, syringic acid, cinamic acid and (+) - catechin.

Based on these results, the pomaces from the Feteasca Neagra and Cabernet Sauvignon cultivars are recommended as sources of polyphenols and several phenolic compounds with great potential for application in food industry as functional ingredients in food products or in food supplements.

These compounds can be used in preserving human health or as an additional treatment of several diseases alongside with the pharmaceutical treatment prescribed.

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